

**Studies on some Activities
of the Male Accessory Glands, Especially
the Production of Male Sperm Antagglutin,
and their Relations to Fertility**

BY

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ALMQVIST & WIKSELLS BOKTRYCKERI AB

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THESIS

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PREFACE

The present paper is a summary of the following communications, which will be referred to by the numbers (24-29) given in the list of references at the end of this summary.

- I. J. E. Kihlström, The Effect of Artificial Light upon the Production of Male Sperm Antagglutin in Rabbits. *III. International Congress of Animal Reproduction, I*, 17-18, 1956.
- II. J. E. Kihlström, Effect of Artificial Light upon Male Sperm Antagglutin Production. *Fertility and Sterility, 9*, 114-119, 1958.
- III. J. E. Kihlström, On some effects of light upon the activity of the male accessory glands in rabbits. *Arkiv för Zoologi, 11*, 379-382, 1958.
- IV. J. E. Kihlström, On the Influence of Frequency of Ejaculation upon Some Activities of the Male Accessory Glands in Bulls and Rabbits. *Acta Zoologica, 39*, 217-226, 1958.
- V. J. E. Kihlström, On Seasonal Variations in the Production of Male Sperm Antagglutin and its Relation to Fertility in Bulls and Rabbits. *Acta Zoologica, 39*, 259-275, 1958.
- VI. J. E. Kihlström, Studies on the hormonal regulation of male sperm antagglutin production in rabbits. *Arkiv för Zoologi, 11*, 575-582, 1958.

The investigations have been performed at the Institute of Zoophysiology, University of Uppsala, during the years 1954-1958.

To my teacher in zoophysiology, Professor Per Eric Lindahl, who introduced me into this field of research, I have pleasure in expressing my sincere gratitude for his active interest, steady encouragement, and support throughout my work.

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INTRODUCTION

Ejaculated mammalian spermatozoa have a tendency to agglutinate head to head. According to Yamane (55) agglutination is brought about by the action of inorganic ions in the diluter. The same opinion is maintained by Milovanov and Selivanova

(47, quoted from 3) who found agglutination of ram spermatozoa to occur when semen is diluted more than 32 times. Other investigators have ascribed agglutinability to cessation of the motility (30). It must be mentioned, however, that in some cases this phenomenon is observed also immediately after ejaculation, while the spermatozoa are still highly motile. According to the opinion of Lindahl and Nilsson (40) the spermatozoa will agglutinate only in a special state of maturity. Kato (22) and Lindahl (31) have pointed out that the agglutinability or "stickiness" of the surface of the spermatozoan head may be of importance in the processes leading to the union of the spermatozoa with the eggs.

Agglutinated spermatozoa show disturbed motility which most likely diminishes the probabilities of the spermatozoa to reach the ova. At artificial insemination agglutinated spermatozoa show reduced fertility (3). It could therefore be expected that mechanisms counteracting the tendency towards agglutination exist in the seminal fluid. Kato (22) studied the agglutination of mammalian spermatozoa and concluded "*that some antiagglutinin chemism exists in the sperm serum*". His studies also indicated that this antiagglutinin substance must be produced in the prostatic gland and/or in the epididymis. Lindahl and Kihlström (34) found agglutinated spermatozoa to be dispersed at addition of fresh sperm plasma, and these authors proved the existence of an antiagglutinin substance in the prostatic secretion of cattle, swine, rabbit, and man. The biological activity of this principle is confined to the reduced form which adheres to the surface of the spermatozoa. By oxidation the activity is lost, and the substance released from the cells (35). The antiagglutinin property of the seminal fluid was later on observed also by Blackshaw (6).

Lindahl and Kihlström (34) reported the existence of a similar agent also in the reproductive organs of female cattle. The two antiagglutinin factors were named antagglutins. Later on the terms male and female sperm antagglutin were introduced in order to distinguish between the antagglutins originating from either sex (39). The male sperm antagglutin is a proteid which has been isolated, purified, and partly chemically characterized by Lindahl and co-workers (34, 35, 23, 32, 31, 37).

During the last years the present writer has studied the occurrence of male sperm antagglutin from a biological point of view. Thereby the variations of the antagglutin production have been compared with those of some other activities of the accessory glands. The results of these studies are summarized in the present paper.

MATERIAL AND METHODS

In this investigation semen from 25 albino rabbits (24-29) and 32 bulls of the Swedish Red Breed (27-28) has been studied.

The bull semen has been obtained from the A. I. Association at Eskilstuna. All ejaculates yielded by the bulls owned by this Association from Jan. 5, 1956 to Jan. 4, 1957 have been included into the investigation. The material thus obtained has the great advantage of permitting studies of possibly existing correlations between the properties of semen and the fertilizing capacity of the individual ejaculates.

For the purpose of collecting semen the bulls were allowed to mount a cow. The ejaculates were then discharged into an artificial vagina handled by the veterinarian. With a few exceptions the bulls were put to service regularly every seventh day, and generally two, or in a few cases three ejaculates were obtained from the same bull within thirty minutes. By measuring the volumes and observing density, mass-

activity, and motility the quality of the ejaculates was assessed by the veterinary staff. If considered of good quality the ejaculates yielded by the same bull on the same occasion were pooled before being used for artificial insemination. All the bulls were apparently in good health, they were given the same food, had the same opportunities of physical exercise, and were kept under identical conditions in the same bull stable.

When collecting semen from rabbits an artificial vagina was kept in the hand, and the arm covered with a skin from a female rabbit. After a period of training most males willingly mounted the skin, and ejaculated into the vagina. A few males, however, mounted the skin only if stimulated by the presence of a female rabbit within the cage. In one series of experiments (27) the frequency of semen collection was varied from once a day to once every twelfth day, in other experiments (24-26) the intervals between the collections were 3-5 days, and in a few cases as much as 7 days. When studying the hormonal regulation of the production of antagglutin (29) semen was collected once a day. The figures included in the study of the seasonal variations in the production of antagglutin in rabbits (28) finally are based upon analyses of semen yielded by the rabbits with intervals of 3-4 days between the ejaculations. All rabbits used have been identically tended, and have not been observed to suffer from any diseases during the experimental time.

All semen samples have been rapidly frozen (24-25) by dipping the tubes into mercury at a temperature of -18°C , and then stored at this temperature until analysed.

Male sperm antagglutin is produced in the prostate, and the amounts of antagglutin in the semen can thus be considered as reflecting the activity of this gland. The determinations of the amounts of antagglutin in the samples have been carried out according to the method of Lindahl, Kihlström, and Ross (38), with two slight modifications (28). According to these authors the standard deviation calculated from twenty duplicate determinations on bull semen in the range 0.5 to 3.5 μg antagglutin is 0.103 μg . With the exception of the first investigation (24-25) the determinations have usually been performed with 0.2 ml of seminal fluid. In rabbits the average amount of antagglutin in this volume is about 1.6 μg , and the average error of the determinations is thus about 7 per cent. The corresponding figures for bull semen are 1.2 μg and 8 per cent, respectively.

The accuracy of the determinations performed further depends upon the purity of the preparations of antagglutin used for calibrating the method of determination. The preparations have been freed from small molecules by dialysis, and paper electrophoretic studies reveal only one protein component (35). Besides, all preparations have been carried out in exactly the same manner. This justifies the assumption that the proportion of impurities is nearly constant in different preparations. This being the case the impurities of the preparations will certainly influence neither the results obtained nor the conclusions drawn. The figures given, however, might thus possibly have to be multiplied by a factor smaller than one.

The antagglutin adhering to the surface of the spermatozoa in fresh semen has been released by oxidation with hydrogen peroxide (35, 38, 24, 25). In order to determine whether the concentrations used (25) were large enough to release all antagglutin from the cells, six preparations with the same amount of semen but with varying concentrations of hydrogen peroxide were performed. The results are recorded in Table I.

In another series of experiments the effect of freezing the semen upon the results

Table I

Concentrations of hydrogen peroxide	0	4×10^{-3} M	8×10^{-3} M	16×10^{-3} M	4×10^{-2} M	8×10^{-2} M
Amounts of antagglutin obtained	0.80 μ g	1.60 μ g	1.52 μ g	1.60 μ g	1.60 μ g	1.60 μ g

of the determinations was studied. Five different samples of semen were each divided into two portions. In the one portion the amount of antagglutin was immediately determined; the other portion was frozen, and its content of antagglutin determined after storage of the sample for a week at -18°C . In all cases the values obtained from the two samples in such a pair were nearly identical.

It was impossible to analyse all samples as rapidly as they were obtained, and a number of samples were stored in a frozen state for about one year. The concentrations of antagglutin obtained from the analyses of one hundred of such ejaculates have been compared with the concentrations found in the same number of ejaculates stored in the refrigerator for a few days only. In both cases the ejaculates were evenly distributed throughout the year. The results are given in Table II. Thus the length of the time of storage does not influence the amounts of antagglutin determined.

Table II

μ g of antagglutin per ml	Ejaculates stored for a few days	Ejaculates stored for about a year
	5.18 ± 0.775	5.74 ± 0.837

In bulls the prostate and the glands of Cowper are small structures. The main part of the seminal fluid is produced by the noticeably great seminal vesicles (44). Besides, the bulk of the seminal fructose is derived from the latter glands. Thus we can look upon the volumes of the ejaculates and the contents of fructose and other solids in the seminal fluid of bulls as giving information about the activity of the seminal vesicles.

Rabbits, on the other hand, lack seminal vesicles, and the organs known as seminal glands (*glandula seminalis* and *glandula vesicularis*) are regarded as the homologous structures (9). In these animals the prostate, the seminal glands, and the glands of Cowper are of approximately the same size. Moreover, the seminal fructose is produced in the prostate as well as in the seminal glands. Consequently the volumes of the ejaculates and the contents of total solids and fructose in rabbit semen must be regarded as giving information about the total activity of the accessory glands.

The volumes of the ejaculates have been measured in graduated test tubes, and the values recorded with an accuracy of 0.1 ml (bulls) or 0.05 ml (rabbits). The mean volume of bull ejaculates is 5.3 ml, and that of rabbit ejaculates 0.7 ml. The average errors of the determinations are thus about 2 and 7 per cent, respectively. By weighing about 0.1 ml of seminal fluid with an accuracy of 0.02 mg., drying the sample over phosphorpentoxide under vacuum, and weighing again the concentration of solids has been obtained, and expressed in per cent weight per weight. The average

amounts of solids in the samples weighed are 11 mg in bulls and 4 mg in rabbits. Thus in both animals the average error of the determinations is less than one per cent. In a number of ejaculates the amounts of seminal fructose have been ascertained by the method worked out by Roe (50) and modified by Gropper (17). This method gives an error of estimate of about 2 per cent (50). The semen samples having been stored at -18°C , the amounts of fructose lost through the metabolic activity of the sperm cells during storage at this low temperature will certainly not influence the results obtained.

In bull semen the estimations of density, mass-activity, and motility performed by the veterinary staff at the bull station also give information about the activities of the sexual glands. Density and mass-activity were estimated according to a three-graded scale, and motility according to a four-graded scale. In some cases also smaller differences between these steps have been recorded. Because of the subjective character of the estimations, no attention has been paid to these finer gradations.

STATISTICAL TREATMENT OF THE DATA OBTAINED

Most statistical methods are based upon normally distributed populations. Conclusions drawn from statistical treatment are thus reliable only provided that the distribution of the data studied does not deviate too much from the normal curve. In the investigations summarized in this paper three main properties of the ejaculates have been studied, viz. volume, content of antagglutin, and content of solids. 100–200 ejaculates from bulls and rabbits have been selected at random, and the distributions of the values of volume, content of antagglutin, and content of solids studied. After grouping the figures into appropriate classes, histograms were drawn, and the curves of the corresponding normal distributions calculated (See Fig. 1). A comparison by means of the χ^2 -analysis reveals that in bulls as well as in rabbits the distributions of the volumes of the ejaculates do not differ significantly from those of the corresponding normal populations (See Fig. 1). The same holds true for the amounts of solids per ejaculate in bulls.

In order to get as reliable information as possible about the remaining three distributions deviating significantly from the corresponding normal curve the number of ejaculates studied was in these cases increased to about three hundred.

The skewness of the distributions found has been calculated according to the method devised by Dahlberg (8). The values found ranged from +0.10 to +0.59 (See Fig. 1). Of course, no sharp limit can be drawn, but the skewness ought not to be greater than 0.5 when using statistical methods based upon the probability curve (8). Four of the distributions obtained show values of skewness lower than 0.5, and the two remaining values do not appreciably exceed this limit. It may also be pointed out that the validity of the error of the mean as calculated from the standard deviation is comparatively slightly affected by skewness (8). Thus conventional statistical methods, such as the analysis of variance, the t-test, and the correlation analysis are applicable to the material studied. The results obtained from the statistical treatment have been estimated according to the tables published by Hald (18).

The number of inseminations per ejaculate or pool of ejaculates necessary for obtaining an index of fertility which can be considered reliable is discussed in paper number 28. More than twenty inseminations will obviously give a good estimation of the fertility of the semen.

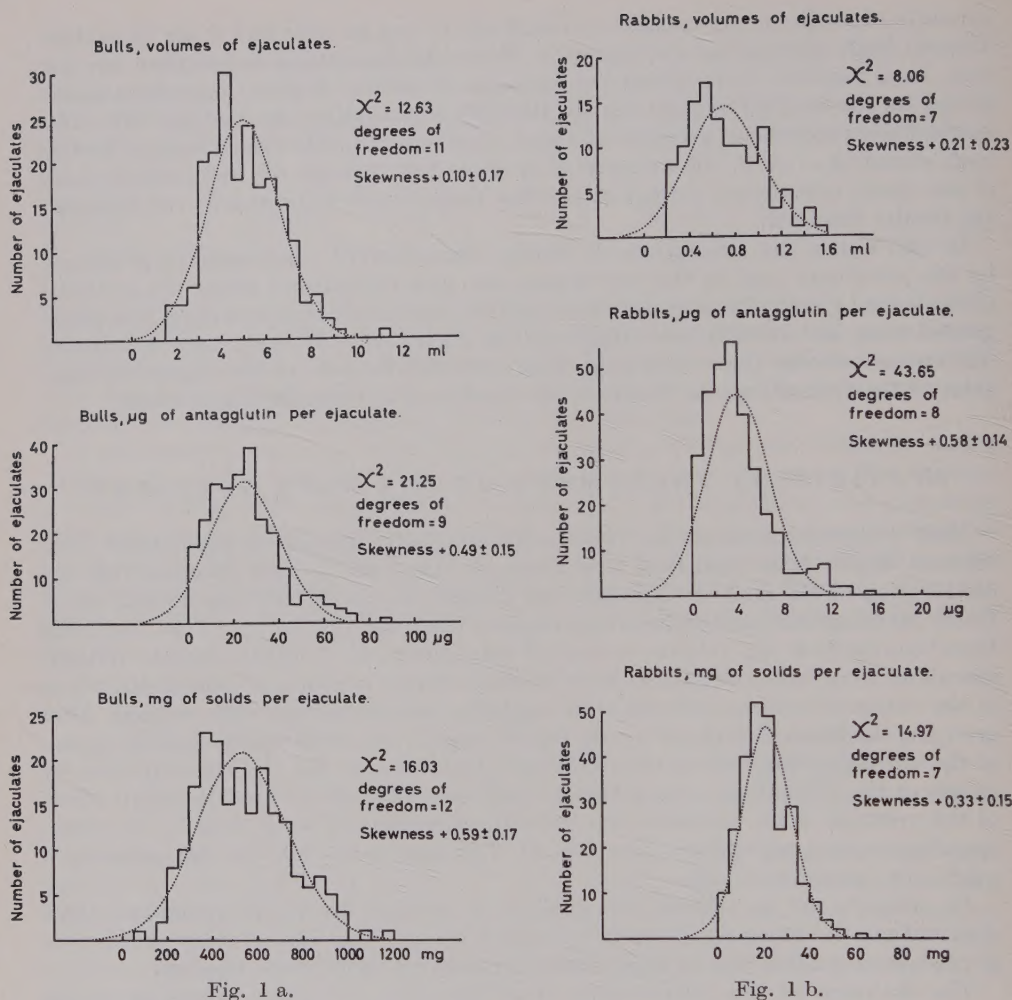


Fig. 1 a.

Fig. 1 b.

SEASONAL VARIATIONS, A DESCRIPTION

When preparing antagglutin from bull semen it was observed that the yield was often larger during summer than during winter. Thus seasonal variations in the production of antagglutin occur. These variations have been studied in bulls and rabbits (28).

Bulls. Many research workers have studied seasonal variations in the semen production of bulls. In this respect, however, different breeds show great differences, and the results obtained are furthermore strongly influenced by the climate. (See for instance 2, 3, 4, 15, 11). According to findings of the present author the volumes of the ejaculates as well as the contents of solids in the sperm plasma are at a minimum

in February, and at a maximum during late summer and autumn (28). The production of antagglutin varies in exactly the same manner. All characteristics studied show significant differences between the mean values obtained during periods of maximum and minimum, respectively (28). The male accessory glands are thus most active during late summer and early autumn. This high activity influences the properties of the semen and thereby the synchronously varying fertility of the bulls (28). Thus the present author has found the fertility to be at a minimum in February, and at a maximum in September. These findings are in agreement with the figures given by Swensson (53). It may be pointed out, however, that the monthly mean values obtained by the latter writer are subject to a remarkably great variability not found by the present author.

Rabbits. It is a fact, well known to practical breeders, that rabbits show low sexual activity while moulting, and seasonal periodicity in the reproduction has been noted by several writers (19, 20, 41, 45, 51, 4, 14, 15). These investigators agree that the optimum season for reproduction in rabbits is approximately from April to June. From July to September, on the other hand, only few female rabbits are willing to copulate, and relatively few of the matings performed are fertile.

According to the present author the male accessory glands in rabbits show low activity approximately from May to June and from August to September, while the activity is high during winter and in the middle of the summer (28).

During the periods of low activity of the accessory glands the male rabbits exhibit a feeble libido (28) and apparently also a low production of sperm cells. Thus Macirone and Walton (42) and Frölich (14) have found a low concentration of spermatozoa in the semen during autumn. Besides, the initial motility of the sperm cells as well as its duration at storage are at a minimum during July and August (14).

For the early part of the year, on the other hand, Macirone and Walton (42) and Frölich (14) found high concentrations of spermatozoa in the semen. The high activity of the accessory glands during winter, approximately from November to April (28), thus coincides with a period of intense spermatogenesis.

All properties studied by the present author show significant differences between the mean values obtained from periods of maximum and minimum, respectively (28). The seasonal variations of the different characteristics, however, are not quite synchronous, possibly indicating that they are regulated by different mechanisms.

The fact that bulls show only one seasonal maximum in the activity of the sexual glands, whereas in rabbits there is one maximum during winter and one in the middle of the summer is most likely connected with other patterns of the reproduction in these animals. Thus it must be emphasized that the cow has a long period of gestation and consequently only one partus per year, whereas the rabbit doe under favourable conditions produces several litters within a year.

Some factors regulating the seasonal activity of the accessory glands will be discussed later.

FERTILITY AND THE PRODUCTION OF ANTAGGLUTIN

As pointed out in the introduction agglutinated spermatozoa exhibit a reduced motility, most likely lowering the fecundity of the spermatozoa. Consequently it

should be expected that, owing to their antiagglutinin activity, the antagglutins are of great importance in maintaining maximal fertility. Lindahl, Ingelman-Sundberg, et al. (33) have shown that in some sterile women, which according to the ordinary clinical investigations seemed to be normal, no active antagglutinin could be demonstrated. Some observations indicate a close relationship between fertility and the presence of active antagglutinin also in the male secretions. In a number of subfertile and sterile individuals disturbances in the production of active antagglutinin could thus be demonstrated (31).

The seasonal maximum in the production of antagglutinin in bulls mentioned in the preceding chapter (See also 28) coincides with the time of maximal fertility as judged from the results of the inseminations. This fact does, however, not necessarily mean that there exists a causal connection between the high production of antagglutinin and the good fertility obtained. The total productive capacity of the accessory glands, estimated as volumes of the ejaculates and the contents of solids in the sperm plasma, is at maximum during the same season (28). It must furthermore be emphasized that fertility also depends upon the sexual condition of the females.

In order to obtain a true picture of the relationship between fertility and the production of antagglutinin we must study the content of antagglutinin in the individual samples in relation to the results of the inseminations performed with the same semen. It must be pointed out, however, that we are dealing with individual ejaculates in few cases only. If considered to be of good quality the ejaculates yielded by the same bull on one occasion were pooled before being used for artificial insemination.

The relationships between fertility and the production of antagglutinin are subjected to great seasonal and individual variations. In our case it was thus possible to study only bulls, which had been put to service regularly throughout the year without longer pauses. Besides, in order to get reliable indices of fertility most ejaculates of these bulls must have been used for insemination of more than twenty cows (28). Only seven animals out of thirtytwo fulfilled these two conditions, and have thus been made the subject of a more detailed study.

These seven bulls, five of which show correlation between fertility and the production of antagglutinin, have been arranged into three groups. In the first group, A, there exists throughout the year a significant correlation between fertility and the content of antagglutinin in the semen. This group comprises only one bull, that also demonstrates a close correlation between fertility and the mean percentage of solids in the sperm plasma of the ejaculates pooled at artificial insemination. There is, however, no multiple correlation between fertility, content of antagglutinin, and percentage of solids (28).

The four bulls in group B show correlations between fertility and the production of antagglutinin only during part of the year, three of the bulls during spring, and one during autumn. The last mentioned bull reveals a slight tendency towards correlation also during spring. Two of the bulls in this group show no correlations between fertility and the other properties of semen studied, and one bull reveals throughout the year a correlation between the mean volumes of the pooled ejaculates and fertility. In the semen of the fourth bull the correlation between fertility and content of antagglutinin ceases at the beginning of April, and is succeeded in the middle of this month by a correlation between fertility and the mean percentage of solids in the seminal fluid of the pooled ejaculates (28).

Group C finally comprises two bulls that do not show any correlations at all

Table III

Group of bulls	Correlations between the production of antagglutin and fertility	Fertility, per cent	Vol., ml	μ g of antagglutin per ejaculate		Solids mg per ejaculate	
				per ml		per cent	
A	Throughout the year	63.9	5.71	31.60	5.51	654.6	11.58
B	During parts of the year	63.4	4.85	25.26	5.31	536.0	10.92
C	None	59.9	4.94	26.75	5.34	488.3	9.69
Comparisons between mean values		A-B to C: A to B:		A to B:		A to B:	
		χ^2 : 17.96 t : 4.09 $p < 0.0005$ (28)		t : 2.49 f : 484 $p < 0.0005$		t : 3.62 f : 298 $p < 0.0005$	
		A to C:				B to C:	
		t : 3.42 f : 298 $p < 0.0005$				t : 2.15 f : 401 $p < 0.025$	

between fertility and the content of antagglutin in the semen. Both bulls, however, show highly significant correlations between fertility and the mean percentage of solids in the pooled ejaculates. Moreover, in one of these bulls fertility is correlated to the mean volume of the pooled ejaculates (28).

The mean values of the studied properties of semen obtained from these three groups of bulls are given in Table III. In group A we find the highest index of fertility and a productive capacity of the accessory glands which is significantly greater than that of the remaining bulls. The bulls in group C, on the other hand, demonstrate a markedly low production of solids and an index of fertility significantly lower than those of groups A and B.

We must look upon the content of antagglutin as well as upon the other characteristics of semen studied as being determined by a number of processes in the sexual apparatus which are important in maintaining maximal fertility. From this point of view the facts summarized in Table III may be interpreted in the following way. In most of the studied bulls some activities of the seminal vesicles, necessary in maintaining maximal fertility, are subnormal and consequently to a certain degree limiting to the fecundity of the semen. This state of the facts is observed as a correlation between fertility on the one hand and the volumes of the ejaculates and/or the contents of solids in the seminal fluid on the other. We find such correlations together with a low fertility in bulls having a markedly low production of solids and low volumes of the ejaculates (group C). These bulls show no correlation between fertility and the production of antagglutin. Higher amounts of solids in the seminal fluid and larger volumes of the ejaculates indicate a greater productive capacity of the seminal vesicles. This being the case (groups A and B) we find higher indices of fertility, and the functional state of the prostate becomes decisive for the level of fertility, which is indicated by a correlation between fertility and the production of

antagglutin. It must be pointed out, however, that the studied bulls have been selected with a view of fulfilling very high demands on fertility, and severe disturbances in the sexual functions are thus not to be expected.

THE RELATIONSHIPS BETWEEN THE PRODUCTION OF ANTAGGLUTIN AND SOME PROPERTIES OF BULL SEMEN

One of the bulls studied often shows spontaneous agglutination of the sperm cells immediately after ejaculation. In the about 1500 ejaculates obtained from the remaining bulls such agglutination has occurred only five times. Theoretically there are four possible causes for agglutination. Firstly the content of antagglutin in the semen may be reduced, secondly the antagglutin present may appear in an inactive form, thirdly it is possible that semen contains abnormal amounts of agglutinating substances and finally the spermatozoa may be subjected to internal changes increasing the tendency towards agglutination. The contents of antagglutin in these ejaculates are nearly identical with those obtained from the same number of control ejaculates selected at random among 1500 normal ejaculates (28). The agglutination is thus not primarily dependent upon a lack of antagglutin. The ejaculates showing agglutination exhibit, however, smaller volumes and lower contents of fructose and total solids than do the control ejaculates (28).

In bulls the seminal vesicles produce the main part of the seminal fluid including the seminal fructose (44). Thus the small volumes and the low contents of solids obtained most likely indicate disturbances in the activity of the seminal vesicles. The most important reducing agent in bull semen, ascorbic acid, is likewise produced in these organs. Thus we must conclude that in the bull in question the functions of the prostate proceed normally as indicated by the amounts of antagglutin found. The functions of the seminal vesicles, however, are disturbed which among other things may cause a low production of ascorbic acid. This being the case the antagglutin would be oxidized, and thereby also rendered inactive. In the absence of active antagglutin a pronounced agglutination of the sperm cells is then easily brought about. Unfortunately it was not possible to verify this conclusion by the biological test for antagglutin (35) and analyses of the seminal ascorbic acid, as this bull had been slaughtered, when these facts became obvious. It is worth mentioning, however, that in some sterile and subfertile individuals with spontaneous agglutination the antagglutin occurs in the oxidized form (31).

Three properties of the semen have been assessed by the veterinaries, viz. density of the sperm cells, mass-activity (wave-motion), and motility. Different motilities of the sperm cells are connected neither with different levels of antagglutin in the semen (28), nor with different contents of solids in the seminal fluid. Ejaculates showing the highest mass-activity on the other hand demonstrate a significantly higher average amount of antagglutin per ejaculate than those inferior in this respect (28). Neither different amounts of total solids in the seminal fluid, nor different volumes of the ejaculates, however, are connected with different mass-activities. Thus we may conclude that mass-activity is dependent upon some factor in the prostatic secretion, and is not very much influenced by the secretions from the seminal vesicles. In the semen from the two bulls having the lowest fertility and which are recorded in the preceding chapter as group C the mean amount of antag-

glutin is significantly greater in ejaculates with the highest density of sperm cells than in the remaining ejaculates (28). In the other bulls there are no such relationships. In no case have any connections been observed between the density of sperm cells on the one hand and the volumes of the ejaculates and their content of solids on the other.

A number of ejaculates deficient in one or several of the properties discussed above have not been used for inseminations. In these ejaculates the volumes and the contents of antagglutin and of total solids, all show lower values than in control ejaculates selected at random, but the differences are not large enough to be significant (28). Low values have also been obtained from the "normal" ejaculates most closely preceding in time those rejected (28). Besides the mean fertility of these last mentioned ejaculates is significantly lower than that of the control ejaculates (28). Ejaculates of low quality thus indicate disturbances of long duration in the sexual functions of the bull.

A highly significant correlation exists between the amount of solids and that of antagglutin per ejaculate (28). No correlation could, however, be demonstrated between the production of fructose and that of antagglutin. These facts are somewhat surprising as the production of fructose as well as other activities of the accessory glands are known to be regulated by the amounts of circulating testosterone (See e.g. 44). This would *a priori* be expected also for the production of antagglutin. There is, however, some doubt as to the value of the seminal fructose test as an indicator of male sex hormone activity. Thus Tyler (54) points out that in man "*low and high fructose values could be found in the same patients at different times without any significant change in any of the obvious signs of good or poor androgenic function.*"

SOME FACTORS REGULATING THE PRODUCTION OF ANTAGGLUTIN

As demonstrated in a previous chapter the production of antagglutin as well as other activities of the accessory glands in rabbits show one maximum during winter and one in the middle of the summer, whereas in bulls the only maximum appears during late summer to early autumn. Many factors, such as the increasing daylight during spring and the free supply of fresh food during the summer, may be important for the production and maintenance of a high activity of the accessory glands. The present author has shown (24-26) that in rabbits artificial light stimulates the activity of the accessory glands, including the production of antagglutin, and that there is a lag of about two months from the beginning of the irradiation until the effect becomes noticeable. The length of this lag is in good agreement with the assumption that the increasing daylight during spring and summer is one of the causes of the seasonal variations observed. Surprisingly rabbits protected from daylight during summer were affected neither in the production of antagglutin, nor in the volumes of the ejaculates, nor in the total production of solids (26).

Maqsood and Parsons (46) failed to detect changes in the reproductive organs of rabbits after keeping the animals continuously in artificial light or continuously in darkness. It is interesting to compare their results with the experience of the present author, showing that such a short time of additional irradiation as ten minutes a day stimulates the activities of the accessory glands. These observations may possibly

indicate that, in stimulating the reproductive activities, the rhythmic alterations between light and dark periods of different length is more important than the amount of light received.

Light apparently influences the endocrine system of higher animals by modifying the amount or the quality of pituitary hormone secretion. Thus Benoit (5) found a rapid increase in pituitary hormone content of the duck and Abolins (1) observed changes in the histology of the pituitary gland in the guinea pig following upon artificial irradiation. According to Fiske (13) light may cause a greater output of FSH than of ICSH, and in the male changes in the spermatogenesis have therefore usually been considered a measure of the effect caused by the light stimuli applied. Concerning the effect of light upon the male accessory glands, however, very little information is available. The papers referred to (24-26) show that light stimulates the activity of these glands, including the production of antagglutin.

Some observations give information about the effect of fresh food upon the production of antagglutin. The bulls studied were sent on pasture during May, and remained there until the end of September. One of the bulls, however, was returned to the bull-stable on July 3, and from that date onwards received only dry hay. In the semen of this bull the highest amounts of antagglutin were found during July. Afterwards they decreased continuously. The remaining bulls show increasing amounts of antagglutin in the semen during late summer and autumn, and the highest values were found usually during August and September. These facts point to a stimulating effect of grazing upon the production of antagglutin. The effect upon the production of total solids, however, appears to be small. Grazing thus appears specifically to augment the production of antagglutin.

Observations made in connection with other studies (Kihlström, unpublished) indicate that in rabbits fresh food is of minor importance for the stimulation of the production of antagglutin.

In another series of experiments the influence of the frequency of ejaculation upon the activity of the accessory glands, especially the production of antagglutin, was studied (27). In rabbits the volumes of the ejaculates and the production of antagglutin and total solids are at a maximum when the animals ejaculate once every second day. When the period of abstinence is four days, the characteristics studied are at a minimum, but increase again with increasing length of the intervals.

The high activity of the sexual glands found, when the rabbits ejaculate once every two days depends most likely upon sexual impulses evoked by this high frequency of ejaculations. The abundance of these impulses apparently suffices for the production of a marked increase in the output of ICSH from the pituitary augmenting the production of testosterone. The latter hormone in its turn stimulates the activity of the accessory glands. Provided that this hypothesis holds good, there must, however, be a great difference between FSH and ICSH in this respect. Thus the output of sperm cells in the ejaculates is not influenced by frequent ejaculations, but continuously rises with increasing periods of abstinence (27). A frequency of ejaculations greater than once every two days probably further increases the stimulation of the accessory glands, but probably also causes an exhaustion of the stored glandular products. When the period of abstinence is shorter than two days, the amount of the secretion yielded in the ejaculates apparently exceeds the productive capacity of the accessory glands. At intervals of 3-4 days between the ejaculations the sexual stimulation and the amount of circulating ICSH decreases, as suggested by the decrease of the volumes of the ejaculates and their content of

antagglutin. The fact that, in spite of lessening sexual stimulation, the volumes of the ejaculates as well as their contents of antagglutin increase at intervals longer than four days seems to indicate a continuous production of secretions from the accessory glands. These secretions are stored in the sexual tract, until ejaculated or lost to the bladder. Such "resting secretion" was first demonstrated in the non-stimulated prostatic gland of the dog (12), and has been thoroughly studied by Huggins and co-workers (21). As demonstrated by the present author (27) in rabbits the frequency of ejaculations has a greater effect upon the production of antagglutin than upon that of the bulk of the solids. One ejaculation every two days produces maximum amounts per ejaculate as well as maximum concentrations of antagglutin (27). At this frequency we also find maximal amounts of total solids in the sperm plasma. The percentage of solids on the other hand continuously increases with increasing periods of abstinence (27). Studies of the production of fructose and citric acid (See 44) indicate that by augmenting the production of physiologically important principles sexual stimulating is able to cause not only quantitative but also qualitative changes in the secretions of the male accessory glands. The present results would thus indicate that the main part of the solids with the exclusion of antagglutin and possibly some other constituents, are substances the production of which is not stimulated by the male sex hormones.

A study of ejaculates yielded successively during about half an hour gives information about the amounts of seminal constituents stored in the sexual tract (27). In bulls, which have large seminal vesicles containing up to 50 ml of secretory fluid (44), the volumes of the ejaculates are nearly constant or even increase in the course of three successive ejaculations. The content of antagglutin in the semen varies in the same manner. This indicates that either relatively big amounts of antagglutin are stored in the prostate, or that this gland during sexual stimulation continuously produces enough antagglutin to replace the amounts lost at the ejaculations. In rabbits the storing capacity of the sexual tract is low. When rabbits ejaculate successively the volumes of the ejaculates rapidly decrease, whereas the contents of antagglutin are only slightly lowered. These facts may indicate that in rabbits the loss of secretions from the seminal glands limits the number of ejaculations before the products of the prostatic gland are exhausted (27).

Gonadotrophins injected during periods of low sexual activity have been shown to stimulate the production of antagglutin (29). Exogenous testosterone, however, did not significantly affect the antagglutin production (29). This somewhat puzzling fact may indicate that the amounts of testosterone circulating in the intact body are abundant enough to evoke maximal stimulation of the prostatic gland. In these case, however, the results obtained following injections of gonadotrophins should imply that exogenous gonadotrophins not only stimulate the production of testosterone in the testis, but also increase the sensitivity of the target organs to the latter hormone. It therefore seems more likely that exogenous testosterone does not add noticeable to the effects of the endogenous male sex hormones (Cf 54). Administered in high doses testosterone slightly depresses the production of antagglutin. This effect probably depends upon the inhibiting action of great amounts of gonadal hormones upon the output of gonadotrophins from the pituitary. The androgenic action of progesterone upon the production of antagglutin is perceptible in doses too small to increase the volumes of the ejaculates and the total production of solids (29). These results may be compared with those of Price, Mann, Lutwak-Mann (48, 49), who found that doses of progesterone having a pronounced stimulating effect upon the

formation of fructose and citric acid were relatively ineffective in increasing the weight of the organs and their contents of nucleic acid. The injection of oestradiol-benzoate produced no noticeable effects upon the production of antagglutin. This has been interpreted as an effect of the antagonistic effects of testosterone and oestradiol, the level of endogenous testosterone being high enough entirely to suppress the action of oestradiol. Some observations indicate that at least in the rat prolactin may be

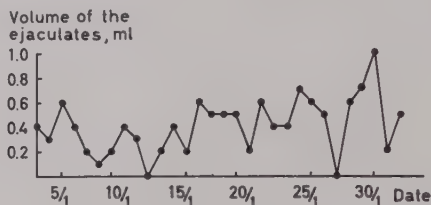


Fig. 2.

important for the normal functions of the prostate (52), and other investigators have found prolactin to augment the action of testosterone (16, 7). According to the present observations, however, prolactin neither alone nor combined with testosterone affects the production of antagglutin or other activities of the accessory glands in rabbits.

In 1956 Doggett (10) observed a marked periodicity in the daily output of sperm cells by rabbits. She found the sperm count to be high one day, and definitely lower on the following one to six days. The average time between peaks in counts was 3.16 days. A similar rhythmic variation in the volumes daily ejaculated by rabbits has been observed by the present author (See Fig. 2). The daily output of solids in the semen was found to vary in the same manner. Moreover, some of the rabbits studied show the same periodicity, though less pronounced, in the amounts of antagglutin ejaculated every day. It may be expected that these phenomena reflect a periodicity in the amounts of sexual hormones circulating in the body. These problems will be analyzed in the future.

GENERAL CONCLUSIONS

Summing up the main results of the investigation we find that the production of antagglutin is regulated chiefly in the same manner as other activities of the accessory glands. Moreover, the amounts of antagglutin in the semen are subject to great seasonal variations, which generally run parallel to those of other properties of the semen, including fertility. In bulls the characteristics studied show one seasonal maximum, appearing during late summer to early autumn. In rabbits we find these characteristics to be at a maximum twice a year, viz, during the winter and in the middle of the summer. A low productive capacity of the seminal vesicles in bulls is shown to be connected with a low fertility. When the seminal vesicles are more active we find a higher index of fertility, and besides the functions of the prostate become determining for the level of fertility as indicated by correlations between fertility and the production of antagglutin.

SUMMARY

The production of male sperm antagglutin and some aspects of its regulation have been analysed in bulls and rabbits, and the results obtained studied in relation to season, properties of the semen, and fertility of the animals.

In bulls the production of male sperm antagglutin as well as the total production of solids, the volumes of the ejaculates and fertility are at a maximum during late summer and early autumn and at a minimum during February.

In rabbits the highest monthly values of the characteristics studied are found during winter, and the lowest during late spring and early autumn with a secondary maximum in the middle of the summer.

Five out of seven studied bulls demonstrate correlations between fertility and the production of antagglutin. In one bull this correlation exists throughout the year. Four other bulls show correlations during part of the year. In the two remaining bulls finally, that have a significantly lower fertility, no correlation between fertility and the production of antagglutin has been found. In some cases there exist correlations between fertility and some of the other properties studied. A further analysis of these relationships indicates that in most of the studied bulls the functions of the seminal vesicles may be subnormal, and thus in a certain degree limit the fertility of the semen. When the volumes of the ejaculates and the contents of solids in the seminal fluid reach higher values, indicating a greater activity of the seminal vesicles, the functional state of the prostate seems to be critical for the level of fertility, as indicated by a correlation between fertility and the production of antagglutin.

Normal amounts of antagglutin were found in ejaculates showing spontaneous agglutination of the sperm cells immediately after ejaculation. There are, however, strong indications that the antagglutin present was oxidized and thereby inactivated due to a lack of the reducing principles in bull semen.

High mass-activities of bull semen are connected with high amounts of antagglutin in the ejaculates. These connections indicate that mass-activity is dependent upon some constituent of the prostatic secretion.

In rabbits light has been shown to stimulate the activity of the accessory glands including the production of antagglutin.

Some observations indicate that at least in bulls fresh food stimulates the production of antagglutin without appreciably influencing other activities of the accessory glands.

The frequency of ejaculations has a great influence upon the output of secretions from the accessory glands. In rabbits the largest ejaculates are yielded, when the interval between the ejaculations is two days. At this frequency we also find the greatest production of antagglutin as well as of other solids in the accessory glands.

A study of ejaculates yielded successively with short intervals indicates that in bulls as well as in rabbits antagglutin is stored in the prostatic gland.

The hormonal regulation of the production of the male sperm antagglutin in rabbits seems to be brought about mainly in the same manner which is well known for other functions of the prostatic gland. A rhythmic variation in the activity of the accessory glands has also been observed.

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